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Original Article

Optimization of Antifungal Activity of *Terminalia catappa* (Combretacae) on the In vitro Growth of *Candida albicans, Aspergillus fumigatus* and *Trichophyton mentagrophytes*

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ARTICLE INFO

Received: 07 Oct 2016
Accepted: 22 Oct 2016

ABSTRACT

Objective: Fungal are responsible for many infectious disease today and continue spreading. The frequency and severity have been growing at an alarming rate for years despite the existence of many drugs. In view of this pathetic situation, it is necessary to develop new active molecule from medicinal plants available and used in the traditional environment. Thus our study on the antifungal activity of *Terminalia catappa* on the *in vitro* growth of *Candida albicans, Aspergillus fumigatus and Trichophyton mentagrophytes* was embarked upon. **Approch experimental:** To achieve this, nine extracts comprising of, two total extracts (aqueous and hydroethanolic 70%), six extracts from liquid-liquid partition of the ethanolic extract and chromatographic fraction F_{12} was made and tested. The tests were carried out on Sabouraud agar double dilution method in slope tubes. **Results and discussion:** All these extract tested are active, more or less pronounced on the three fungal isolates. Among these extracts, the butanolic extract ($X_{3.1}$) was the least active extract and F_{12} fraction the most active extract. Regarding fungal isolates, *Trichophyton mentagrophytes* (MFC=0.5 µg/mL) was the most sensitive germ to F_{12} fraction and *Aspergillus fumigatus* the least sensitive (MFC= 10 µg/mL). As for *Candida albicans*, it was inhibited by F_{12} fraction at a value of MFC = 5 µg/mL. **Conclusion:** The chromatographic extract (fraction F_{12}) is most active extract.

Keywords: *Terminalia catappa,* Antifungal activity

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1. INTRODUCTION

Plants have always been used by humans as good source of food, cosmetics and drugs. From time immemorial they formed the basis of some very developed traditional medical systems.These traditional system of medicine still plays a

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fundamental role to the extent that the World Health Organization (WHO) estimated that 80% of the world population depends on traditional medicine to solve their underlying health problems. And *Terminalia catappa Linne*, pantropical plant is one of the plants used in traditional medicine environment to treat many diseases[1,2,3,4]. In traditional medicine, this species is known for its, antiparasitic, anti-infectious, antihypertensive and antidiabetic properties[5].

The emergence of fungal infections has become a serious public health problem. The fungal species responsible for most infections are *Candida albicans*, they are responsible for most of *candidiasis*[6], *Aspergillus fumigatus*, is a filamentous saprophyte fungus,opportunistic responsible for most fungal infections called pulmonary aspergillosis and extrapulmonary[7]. And *Trichophyton mentagrophytes*, a polymorphic fungus causing ringworms called microïdes, giving a lesion similar in appearance to kerion, difficult to treat[8].

In view of these properties that this plant possesses, this study was initiated to test the antifungal properties shown by this plant and improve to some extent this activity[9].

2. MATERIAL AND METHODS

Plant Material

The plant material is the powder obtained from the bark of *Terminalia catappa* it was coded $TEKAM_3$.

Fungus tested and culture Media

The isolated fungal tested were *Candida albicans*, *Aspergillus fumigatus* and *Trichophyton mentagrophytes* provided by the mycology laboratory of the Training and Research Unit of the faculty of Medical Sciences of the University of Félix Houphouët-Boigny (Côte d'Ivoire). These germs were isolated from patients in the Infectious diseases department of the University Teaching Hospital of Treichville (Côte d'Ivoire).

The Sabouraud agar, acidic pH (5.7) was used for various tests.

Preparation of extracts

The bark of *Terminalia catappa* was cut into pieces dried and was crushed. The powder obtained ($TEKAM_3$) was used to prepare the ethanolic extracts this as follows:

One hundred grams (100 g) of powder was extracted in blender with one liter (1L) Solvent (ethanol 70%). The homogenate obtained was filtered in a square of fabric and successively filtered twice on cotton wool and then Whatman 3mm filter paper. The ethanolic extract (X_0), it was obtained by evaporation to dryness.

Subsequently, three (3) portions of X_0 of 10 g each were formed and separately subjected to a liquid/ liquid partition in 100 mL of 3 different solvents (hexane-water, ethyl acetate-water, butanol-water, 50/50; v/v). After decantation, the various phases were concentrated under vacuum; the following extracts were obtained respectively:

$X_{1.1}$: the hexane phase, $X_{1.2}$: the aqueous phase from the hexane – water partition, $X_{2.1}$: the acetate phase, $X_{2.2}$: the aqueous phase resulting from the partition of ethyl acetate – water, $X_{3.1}$: the butanol phase, $X_{3.2}$: the aqueous phase resulting from the partition butanol – water.

In addition, $X_{1.2}$ extract was then chromatographed on a gel filtration column of Sephadex

G_{25} whose characteristics are: diameter = 1 cm, gel height = 55 cm, flow = 0.22 ml / min.

The fractionation was done with 5 g of the extract $X_{1.2}$. Distilled water was used as eluent and 10 mL samples were taken. Twenty (20) fractions were obtained (F_1 to F_{20}).

Thus all these extracts were tested for biological assays (three germs).

Preparation of culture media

The medium was prepared according to the instructions of the manufacturer's protocol. The inclusion of the plant extracts in the agar was made according to the method of the double dilution agar slopes[10]. Each series of test consisted of 9 tubes containing plant extracts and 2 control tubes. The control tubes are used as control tubes in which one was without a plant

extract used to monitor the growth of germs, and the other germ-free tube and without plant

extract was used as sterility controls to the culture medium. For the 9 test tubes, concentrations ranged from 1560 to 0.5 µg/mL biding by a geometrical reason of ½. All the 11 tubes of each series are sterilized by autoclaving at 121 °C for 15 minutes and then inclined in a storage area to allow for cooling and solidification of the agar.

Antifungal Assay

The different inoculum was prepared from fresh cultures of 3 fungal isolates on inclined agar; 48 hours for *Aspergillus fumigatus* and *Candida albicans*; and 5 days for *Trichophyton mentagrophytes*. Indeed, one to two colonies was removed with a handle and then homogenized in 10 mL of sterilized distilled water (10° suspension, concentrated to 10^6 cells / mL). This has helped to prepare a second suspension 10^{-1} by dilution $1/10^{th}$ by transferring 1 mL of 10° suspenpension in 9 mL of sterilized distilled water. This suspension is concentrated to 10^5 cells/mL.

The culture of different fungal germs was done on previously prepared medium, except in the sterility control tube, by transverse striations seeding of 10 µL (1000 cells) of the suspension 10^{-1} with the aid of a micropipette.

The cultures thus produced, all tubes were incubated in an oven at 30 °C for 48 hours for *Aspergillus fumigatus* and *Candida albicans* and 5 days *for Trichophyton mentagrophytes*.

Moreover, the charges of each inoculum were verified by dilutions of the suspension 10^{-1} to 10^{-2}, 10^{-3} and 10^{-4}.

These tests were repeated 6 times for the extracts X_0, $X_{1.2}$, $X_{2.2}$, $X_{3.2}$ and F_{12} and 3 times for others extracts.

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Counting

After the incubation time, colonies of different fungal organisms were numbered according to the method of GUEDE GUINA *et al.* in 1997[10] by direct counting with a colony counting pen. Growth in the nine (9) tubes of each experimental series was evaluated as a percentage of survival calculated compared to 100 % of survival in the growth control tube. By processing the data obtained during the experiment, we are able to determine the following antifungal parameters: minimum inhibitory concentration (MIC), minimum fungicide concentration (MFC) determined by sterility test of the tube corresponding to the MIC. By plating a sample taken from the surface of agar of MIC tube on a new agar and the concentration for 50 % inhibition (IC_{50}) is determined graphically.

The values of MFC or MIC and where appropriate the IC_{50} allow for the assessment of results between one extracts and the other.

As for the concentration of inoculum, it was determined by the ratio calculation of the factor dilution and the volume sample.

3. RESULTS AND DISCUSSION

After 48 hours and 5 days of incubation according to the germs and at 30 °C, we observed compared to the control tube's contents, a gradual decrease of the number of colonies of different fungal isolates in experimental tubes in line with the increasing concentration of the extracts.

Effective inhibitions were obtained at different concentrations level depending on the extracts. In addition, the table (1) below reflects the values of the antifungal parameters of different extracts: total, partitioned and the most active fraction.

Obtained results showed that these extracts are active on all tested fungal germs. No resistance was observed (table 1). Nevertheless this performance varies according to solvents and in a dose-response relationship.

The aqueous and ethanolic extracts showed good activity on *Candida albicans isolates*[9]. Of these extracts the most active extracts is hydroethanolic extract, based on result it is 2, 4 and 19 times more active on *Candida albicans, Aspergillus fumigatus and Trichophyton mentagrophytes* than aqueous extract. These results are 32 times better than those obtained by Kouakou *et al.* 2007[11] with the aqueous extract of *Thonningia sanguina* (MFC = 1560 µg/mL on *Candida albicans* and 12500 µg/mL on *Aspergillus fumigatus*). Moreover they are better than the results obtained by Thes *et al.*, 2011[12] in *Trichophyton mentagrophytes* (MFC = 390 µg/mL) with a MISCA-MATES soap made from oil extracted from *Miltracarpus scaber*, *Mareeya micrantha* and *Cassia alata*. The activity of the ethanolic extract ($X_{1.2}$) of *Terminalia cattapa* compared to that of the ethanol extract of *Cassia alata* on the *in vitro* growth of *Candida albicans* showed that the extract $X_{1.2}$ is more active, MFC $X_{1.2}$ = 40 µg/mL against 312 µg/ mL[13]. It is also more active than the methanol extract of Chene-liège on *Trichophyton rubrum* and *Candida albicans* with respective MIC 50000 µg /mL and 12500 µg / mL according to the work of Hassikou *et al.* in 2014[14].

Besides the total extract (hydro-ethanolic), the extracts from the partitioned and the F_{12} fraction effect on the *in vitro* growth of these fungal isolates revealed that these extracts also have better activities. That of the F_{12} fraction was more active given the MFC values (table).

Table 1: Antifungal parameters of the different extracts

Extracts	***Candida albicans***		***Aspergillus fumigatus***		***Trichophyton mentagrophytes***	
	IC_{50} (µg/mL)	**MFC (µg/mL)**	**IC_{50} (µg/mL)**	**MFC (µg/mL)**	**IC_{50} (µg/mL)**	**MFC (µg/mL)**
X_0	139	190	14	90	3,8	10
$X_{1.1}$	59	390	177	780	1,4	10
$X_{1.2}$	18	40	6	40	1,3	5
$X_{2.1}$	38	190	28	390	1,8	5
$X_{2.2}$	32	90	25	190	1	10
$X_{3.1}$	500	1560	10	780	1,6	10
$X_{3.2}$	59	190	32	190	1,27	5
F_{12}	1,6	5	1	10	0,04	0,5

Furthermore, regarding the different partitions, the aqueous phases are more active than the organic phases (table 1). That signified that the active ingredient would be concentrated in those phases. The most active extract as a result of partition was the extract from the hexane-water partition (aqueous phase $X_{1.2}$). It is 4.75, 2.25 and 38 times more active respectively on *Candida albicans, Aspergillus fumigatus and Trichophyton mentagrophytes* than its original extract X_0. The activity of the extract $X_{1.2}$ is better than that reported by Agban *et al.*, 2013 [13], with the dichloromethane extract of *Piliostigma thonningii* (MIC = 625 µg/mL) on the *in vitro* growth of *Candida albicans*. The partition of hydro-ethanolic extract in different solvent mixture has contributed in improving the activity of this plant species.

Fractionation of the extract $X_{1.2}$ has produced extracts of which the most active is the extract from the F_{12} fraction (table 1). Analysis of these results showed that this fraction is 8, 4 and 10 times more active than its original $X_{1.2}$ extract on *Candida albicans, Aspergillus fumigatus and Trichophyton mentagrophytes* respectively. The results of the F_{12} fraction is better than those obtained by Kporou *et al.*, 2010[15], of which the most active fractions (F_6, F_7, F_8) from an hexane extract of *Miltracarpus scaber* with a MFC value of 781 µg/mL on the *in vitro* growth of *Candida albicans*.

From our results, the most sensitive fungal isolate is *Trichophyton mentragrophytes* and the least sensitive is *Aspergillus fumigatus*, *Candida albicans* has an intermediate

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sensitivity. According to the MFC values, the most active extract is the F_{12} fraction.

Moreover, we observed that the filtration chromatography on Sephadex G_{25} gel has greatly improved the antifungal activity of *Terminalia cattapa.*

4. CONCLUSION

This study confirms the healing capacity granted to this plant species in traditional medicine. Indeed, all tested extracts have effective antifungal activity coupled with a fungistatic action on the *in vitro* growth of the three fungal isolates. The most active extract is the extract from the F_{12} fraction. *Trichophyton mentagrophytes* appears to be the most sensitive fungal germ and *Aspergillus fumigatus* the least sensitive. The sensitivity of *Candida albicans* is intermediate.

The anti-infective property granted to *Terminalia catappa* is therefore justified. However, further study must be done to determine the chemical structure of the active ingredient with appropriate methods.

5. ACKNOWLEDGEMENT

Our sincere thanks goes to the entire staff of the Mycology Laboratory of the Faculty of Medical Sciences of the University of Felix Houphouet-Boigny (Cote d'Ivoire) for providing fungal germs, the National Floristic Centre for the identification of the plant studied and members of Pharmacodynamics-Biochemical Laboratory of the Faculty of U.F.R. Biosciences of University of Félix Houphouët-Boigny (Cote d'Ivoire).. And members of departments of Biochemistry-Microbiology and Botanic of the U.F.R. Agroforestry, University of Jean Lorougnon Guede Daloa (Cote d'Ivoire).

6. REFERENCES

1. E. J. Adjanohoun et L. Aké-Assi. Contribution au recensement des plantes médicinales de Côte d'Ivoire, CRES. Université Côte-d'Ivoire, Centre National de Floristique, 1979 ; 40-219.
2. L. Aké-Assi. et S. Guindo. Plantes utilisées dans la médecine traditionnelle en Afrique de l'Ouest. Éd. Roches, Paris 1991, 151 pages.
3. A.A. Diafouka, J. Lejoly, Plantes hypotensives utilisées en médecine traditionnelle à Brazzaville (Congo), BARAB Actes du 2ème Colloque Européen d'Ethnopharmacologie et de la 2ème Conférence internationale d'Ethnomédecine, Heidelberg, 24-27 mars 1993. 5 pages.
4. G.K.N. Ahoussou, Abdourahamane Sangaré et Alassane Sangaré**,** Côte d'Ivoire : Rapport de pays pour la conférence internationale de la FAO sur les ressources phytogénétiques, Leipzig, 1996, 75 pages
5. H.M. Burkill,. The useful plants of West Tropical Africa. ed. 2, Royal Bot. Garden Kew 1997; 138-140.
6. Hilbert G. Infections fongiques invasives en réanimation et place de l'anidulafungine dans l'arsenal thérapeutique. Réainimation, 2007 ; 16 : S280-284.
7. Anonyme. Aspergilloses et autres champignons filamenteux opportunistes. Cours de Association Française des enseignants de Parasitologie et Mycologie. Université Médicale Virtuelle Francophone, 2014, 19 pages.
8. A Chollet, V. Cattin, M. Fratti, B. Mignon and M. Monod. Which fungus originally was Trichophyton mentagrophytes? Historical review and illustration by a clinical case. Mycopathologia, 2015; Vol. 180 (1) : 1-5.
9. Ackah J.A.A.B., Kra A.K.M., Zirihi G.N. et Guédé-Guina F. Evaluation et essais d'optimisation de l'activité anticandidosique de Terminalia catappa linne (TEKAM$_3$), un extrait de Combretaceae de la pharmacopée ivoirienne. Bulletin de la Société Royale des Sciences de Liège, 2008; Vol. 77 : 120-136.
10. F. Guédé-Guina, A. K. M. Kra, M. Vangah-Manda et G. M. Bonga, Inhibition par MISCA-F2 de la croissance de *Aspergillus fumigatus, Candida albicans et Cryptococcus neoformans* trois germes opportunistes du SIDA. J. Afrique Bioméd., 1997 ; 2 :11-16.
11. Kouakou A.V., N'guessan J.D., Kra A.K.M. et Guede-Guina F., 2007. Activité antifongique et screening phytochimique de THOS (extrait aqueux de *Thonningia sanguinea*). Revue Med. Pharm. Afr. (20): 45-52.
12. P.M. Thes, I.A. Soumahoro, Ackah J.A.A.B., G.N. Zirihi, A.J. Djaman. Action du savon Misca-mates dans le traitement des teignes. Phytothérapie, 2011; 9: 354–358
13. A Agban, K.A. Gbogbo, Y.P. Hoekou, K. Atchou, T. Tchacondo, K. Batawila, C. De Souza, M. Gbeassor**.** Evaluation de l'activité antifongique des extraits de *Cassia alata* et de *Piliostigma thonningii* (Schumach) Milne Redh.(fabaceae) sur *Candida albicans*. International Journal of Biological and Chemical Sciences, 2013, Vol. 7 (3): 1041-1047.
14. R. Hassikou, H. Oulladi, M. Arahou. Activité antimycosique des extraits de Cheine-liège Quercus suber sur *Trichophyton rubrum* et *Candida albicans*. Phytothérapie, 2014; Vol. 12 (4) : 206-212.
15. K.E. Pkorou, A.K.M. Kr, S. Ouattara, F. Guede-Guina, A.J. Djaman. Amélioration par fractionnement chromatographique de l'activité anticandidosique d'un extrait hexanique de Miltracarpus scaber Zucc. sur la croissance in vitro de Candida albicans et candida tropicalis. Phytothérapie, 2010; Vol. 8 (5) : 290-294.

Conflict of Interest: None

Source of Funding: Nil

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